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Chromosome Numbers of Six Species of *Hydrocotyle* (Umbelliferae) in Japan

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Chromosome numbers of *Hydrocotyle* occurring in Japan were counted for 226 plants representing the following six species: *H. dichondroides*, *H. javanica*, *H. maritima*, *H. ramiflora*, *H. sibthorpioides* and *H. yabei*. In these, the four species of *H. dichondroides*, *H. ramiflora*, *H. sibthorpioides* and *H. yabei* occurred as diploids (2n = 24), and *H. javanica* as an octoploid (2n = 96), while *H. maritima* showed euploid variations of hexaploids (2n = 72), octoploids (2n = 96) and nonaploids (2n = 108). New counts were reported for *H. maritima* (2n = 72, 96, 108) and *H. yabei* (2n = 24). All had a common basic chromosome number of x = 12 within the four basic chromosome numbers of x = 8, 9, 11 and 12 known in the genus.

Key words: Chromosome number, *Hydrocotyle*, Hydrocotyloideae, polyploidy, Umbelliferae.

Hydrocotyle L. belonging to the Umbelliferae subfam. Hydrocotyloideae comprises about 130 species of creeping perennial herbs (Mabberley 1997). In Japan seven species of *Hydrocotyle* are reported (Ohba 1999). Chromosome number is poorly studied. However, the reported chromosome numbers for the taxa in *Hydrocotyle* are greatly variable as the following: 2n = 18, 22, 24, 32, ca. 36, 48, 44, 48, 64, 72, 96 and ca.160 (Fedorov 1969). Our investigation aims to increase the knowledge on the chromosomes of *Hydrocotyle* and deals with the somatic chromosome numbers of the six species *H. dichondroides*, *H. javanica*, *H. maritima*, *H. ramiflora*, *H. sibthorpioides* and *H. yabei* that occur in Japan.

Materials and Methods

Plant materials of the following six species of *Hydrocotyle* occurring in Japan: *H. dichondroides* Makino, *H. javanica* Thunb., *H. maritima* Honda, *H. ramiflora* Maxim., *H. sibthorpioides* Lam. and *H. yabei* Makino, collected from the wild populations, were used (cf. Appendix). These were grown at the experimental garden of University of Toyama. Their newly formed root tips harvested from the potted plants were pretreated in a 2.2 mM 8-hydroxyquinoline solution for an hour at 25°C and subsequently kept for about 15 hours at 6°C. The root tips were fixed in freshly mixed Carnoy's fixative (3 : 1 ethyl alcohol: acetic acid) for 1 hour, soaked in 1N HCl for a few hours, and macerated in 1 N HCl at 60°C for

about 10 minutes. After being immersed in tap water, their meristems were stained in a drop of 1.5 % lacto-propionic orcein on the slide glass and usual squash technique was applied for the examination of somatic chromosome numbers. Voucher specimens are deposited in the Herbarium of Department of Biology, Faculty of Science, University of Toyama.

Results and Discussion

1. *Hydrocotyle dichondroides* Makino

Chromosome count was determined for one plant, which showed 2n = 24 chromosomes (Fig. 1, Appendix). This chromosome count agrees well with the earlier meiotic count of n = 12 reported by Hsu (1968), and different from the meiotic count of n = 11 by Liu (1961).

2. *Hydrocotyle javanica* Thunb.

A chromosome count of 2n = 96 was obtained from all the five plants examined (Fig. 2, Appendix). This chromosome count corresponded to that reported by Liu (1961), and was different from the counts reported previously of n = 21, 25, 26, 27 (Hore 1976, sec. Goldblatt 1981), 36 (Sharma 1970 (sec. Moor 1973), Krishnappa and Basappa 1988), 46 (Sarkar et al. 1982), 48 (Liu 1961) and 84 (Constance et al. 1971) and 2n = 18 (Subramanian 1986), 36 (Datta and Maiti 1968, sec. Moor 1973) and 72 (Sharma and Bhattacharyya 1959, Krishnappa and Basappa 1988).

3. *Hydrocotyle maritima* Honda

A total of 106 individuals collected from 81 localities in Toyama Pref., central Japan, were studied. Out of the 106 individuals, 29 plants (27. 6 %) had 2n = 72 chromosomes (Fig. 3A, Appendix), 73 plants (69.5 %) had 2n = 96 chromosomes (Fig. 3B, Appendix), and four plants (2.9 %) had 2n = 108 chromosomes (Fig. 3C, Appendix). Figure in parenthesis shows the frequency.

These chromosome counts were different from the first count of n = 12 for this species by Sun et al. (1996, as 12II).

4. *Hydrocotyle ramiflora* Maxim.

A total of 91 individuals collected from 66 localities in Hokkaido and Honshu were studied. All had 2n = 24 chromosomes (Fig. 4, Appendix). This chromosome count was consistent with the previous report by Pimenov et al. (1996, as n = 12 + 1-3B).

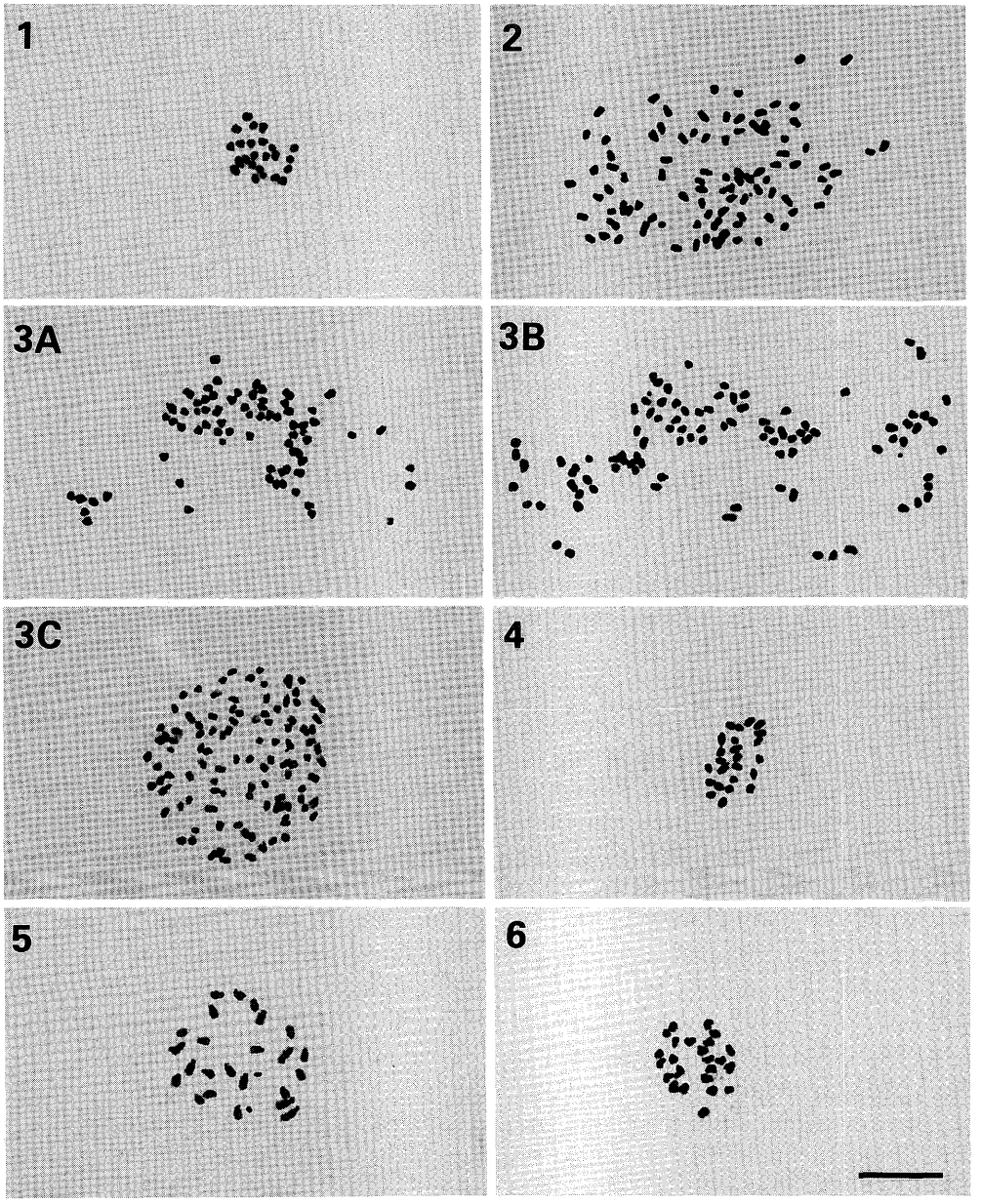
5. *Hydrocotyle sibthorpioides* Lam.

A chromosome count of 2n = 24 was obtained from all the plants studied (Fig. 5, Appendix). In the complement, two chromosomes had satellites. The chromosome count was consistent with that reported by Sharma and Bhattacharyya (1959, under the name of *H. rotundifolia*), Liu (1961, as n = 12), Hsu (1967), Hsu (1968, as n = 12), Sharma (1970, sec. Goldblatt 1981, under *H. rotundifolia*, as n = 12), Hore (1976, sec. Goldblatt 1981, under *H. rotundifolia*, as n = 12), Constance et al. (1976, as n = 12), Ahmad and Koul (1980), Cauwet-Marc et al. (1980), and Hamal et al. (1986), and different from those reported by Subramanian (1986, 2n = 18, under *H. rotundifolia*), Krishnappa and Basappa (1988, n = 9 and 2n = 18, under *H. rotundifolia*), and Borgmann (1964, 2n = 48).

6. *Hydrocotyle yabei* Makino

A chromosome count of 2n = 24 was obtained from all the 19 individuals collected from 18 localities (Fig. 6, Appendix). The first cytological study for this species by Sun et al. (1996, as *H. japonica* Makino) reported 2n = ca. 96 chromosomes. Thus our count was the first record in *H. yabei*.

Hydrocotyle is known to be a polybasic genus having x = 8, 9, 11 and 12 (Darlington and Wylie 1955, Hsu 1986). The present chromosome study of six species of Japanese *Hydrocotyle* disclosed that they had a varia-



Figs. 1–6. Somatic chromosomes of *Hydrocotyle* of Japan. 1: *H. dichondroides* (2n = 24). 2: *H. javanica* (2n = 96). 3A: *H. maritima* (2n = 72). 3B: *H. maritima* (2n = 96). 3C: *H. maritima* (2n = 108). 4: *H. ramiflora* (2n = 24). 5: *H. sibthorpioides* (2n = 24). 6: *H. yabei* (2n = 24). Scale bar indicates 10 μm.

tion of 2n = 24, 72, 96 and 108 chromosomes. Among the four basic chromosomes numbers of x = 8, 9, 11 and 12 proposed by Darlington and Wylie (1955) and Hsu (1968), x = 12 is the most suitable basic chromosome number of these species. For this reason, x = 12 is a divisor of all the chromosome counts found in the present study.

The chromosome counts of 2n = 24, 72, 96 and 108 found in the present study are, thus, judged to be diploid (2n = 24), hexaploid (2n = 72), octoploid (2n = 96) and nonaploid (2n = 108), respectively.

This study revealed that *H. maritima* in Japan was composed of hexaploids with 2n = 72, octoploids with 2n = 96 and nonaploids with 2n = 108 chromosomes. The nonaploid (2n = 108) plant was very rarely found compared with the hexaploids (2n = 72) and octoploids (2n = 96). The nonaploid seems to be produced by fusion between a haploid gamete (n = 36) and an unreduced gamete (n = 72) arisen spontaneously from a hexaploid plant with 2n = 72 chromosomes.

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岩坪美兼，松田美紀*，笹村和幸**，鳴橋直弘：

日本産チドメグサ属6種の染色体数

チドメグサ属は世界に約130種（Mabberley 1997），わが国には7種が知られている（Ohba 1999）．タカサゴノチドメを除くわが国の6種について染色体の観察を行ったところ，染色体数はケチドメ（*H. dichondroides*, 2n = 24），オオバチドメ（*H. javanica*, 2n = 96），ノチドメ（*H. maritima*, 2n = 72, 96, 108），オオチドメ（*H. ramiflora*, 2n = 24），チドメグサ（*H. sibthorpioides*, 2n = 24），およびヒメチドメ（*H. yabei*, 2n = 24）であった．ノチドメの 2n = 72, 96, 108とヒメチドメの 2n = 24 は，それらにおいて報告されていない新しい染色体数であった．チドメグサ属の染色体基本数には x =

8，9，11，12（Darlington and Wylie 1955, Hsu 1986）が知られている．わが国のこれら6種で観察された染色体数は，x＝12を共通の基本数とする二倍体（2n＝24），六倍体（2n＝72），八倍体（2n＝96），それに九倍体（2n＝108）であった．

ノチドメは，富山県産の個体について観察を行ったところ，六，八，九倍体の種内倍数性が存在することが判明した．観察を行った106個体のうち，九倍体ノチドメは4個体（2.9％）だけであった．九倍体ノチドメは，六倍体ノチドメ（2n＝72）の正常な還元配偶子（n＝36）と偶発的に生じた非還元配偶子（n＝72）の間の受精によって出現したものと推定される．

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Appendix

Chromosome numbers, collection localities, and number of individuals (in parentheses) of studied taxa in Japanese *Hydrocotyle*

H. dichondroides

2n＝24 **Kagoshima Pref.**: Sumiyoshi, Nisinoomote City (1)

H. javanica

2n＝96 **Ishikawa Pref.**: Azumi, Shika-machi, Hakui-gun (1). **Shizuoka Pref.**: Tadaki, Mikkabi-cho, Inasa-gun (1). **Mie Pref.**: Matsusita, Futami-cho, Watarai- gun (1). **Kagoshima Pref.**: Shiroyama, Kagoshima City (1); Uenodan, Kokubu City (1)

H. maritima

2n＝72 **Toyama Pref.**: Maezawa, Kurobe City (1); Nakano, Namerikawa City (1); Nofusue, Nanto City (1); Saimyo, Nanto City (1); Haniu, Oyabe City (1); Iwaodaki, Oyabe City (1); Minetsubono, Oyabe City (1); Funakura, Toyama City (1); Gofuku, Toyama City (1); Hirasawa, Toyama City (1); Hirabayashi, Toyama City (1); Ichidani, Tonami City (1); Iwasekoshinmachi, Toyama City (1); Katakake, Toyama City (2); Kitadani, Toyama City (1); Ishigaki, Uozu City (1); Ishigakidaira, Uozu City (1); Kanayamadani, Uozu City (1); Kurodani, Uozu City (1); Shimajiri, Uozu City (1); Tozo, Uozu City (1); Yokomakura, Uozu City (1); Gomado, Kamiichi-machi, Nakaniikawa-gun (3); Mushidani, Tateyama-machi, Nakaniikawa-gun (1); Teratsubo, Tateyama-machi, Nakaniikawa-gun (1); Aoshima, Nyuzen-machi, Simoniikawa-gun (1)

2n＝96 **Toyama Pref.**: Ajikawa, Himi City (1); Hiragawa, Himi City (4); Isobe, Himi City (1); Tamomi, Kurobe City (1); Fukumitsu-Habu, Nanto City (1); Fukumitsu-Higashitono, Nanto City (1); Fukumitsu-Horinji, Nanto City (1); Fukumitsu-Kaji, Nanto City (1); Fukumitsu-Yomegane, Nanto City (2); Izemi, Nanto City (1); Jabami, Nanto City (1): Kawarasaki, Nanto City (2); Kozu, Nanto City (1); Ohgaya, Nanto City (1); Oki, Nanto City (2); Minotani, Nanto City (1); Shimonashi, Nanto City (1); Dotsubono, Oyabe City (1); Hasunuma, Oyabe City (1); Hirazakura, Oyabe City (1); Ishisaka, Oyabe City (1); Kitaichi, Oyabe City (1); Matsunaga, Oyabe City (1); Matsuo, Oyabe City (1); Suetomo, Oyabe City (1); Tanada, Oyabe City (1); Ushirodani, Oyabe City (1); Fukuokamachi-Shimomukuta, Takaoka City (1); Akamedani, Toyama City (1); Ashu, Toyama City (1); Fuchumachi-Tomisaki, Toyama City (1); Fuchumachi-Tomosaka, Toyama City (1); Fuchumachi-Yasuda, Toyama City (1); Gofuku, Toyama City (2); Hirabayashi, Toyama City (1); Hirasawa, Toyama City (1); Jike, Toyama City (1); Joyama,Toyama City (1); Ohshimo, Toyama City (1); Okuda, Toyama City (1); Shinden, Toyama City (1); Tomisaki, Toyama City (1); Tsukiokamachi, Toyama City (1); Sannokuma, Toyama City (1); Yasuda, Toyama City (1); Higashiyama, Uozu City (1); Ishigaki, Uozu City (1); Ishigakidaira, Uozu City (1); Kanayamadani, Uozu City (1); Kannondo, Uozu City (2); Tozo, Uozu City (2); Oiwa, Kamiichimachi, Nakaniikawa-gun (1); Shakusenji, Kamiichi-machi, Nakaniikawa-gun (1); Tachi, Kamiichi-machi, Nakaniikawa-gun (1); Izumi, Tateyama-machi, Nakaniikawa-gun (3); Mushidani, Tateyama-machi, Nakaniikawa-gun (1); Kanayama, Asahi-machi, Shimoniikawa-gun (1); Sakai, Asahi-machi, Shimoniikawa-gun (1); Tonomachi, Asahi-machi, Shimoniikawa-gun (1); Aoshima, Nyuzen-machi, Simoniikawa-gun (1); Funami, Nyuzen-machi, Simoniikawa-gun (1); Yoshiwara, Nyuzen-machi, Simoniikawa-gun (1)

2n＝108 **Toyama Pref.**: Minotani, Nanto City (1); Nishiakaomachi, Nanto City (1); Baba, Takaoka City (1); Fukuokamachi-Shimomukuta, Takaoka City (1)

H. ramiflora

2n＝24 **Hokkaido Pref.**: Otoecho, Fukagawa City (1); Urausu-cho, Kabato-gun (1); Kuriyama-cho, Yubari-gun (1). **Aomori Pref.**: Sebedi, Yomogita-mura, Higashitsugaru-gun (1); **Toyama Pref.**: Fukuhira, Kurobe City (1); Oritate, Kurobe City (1); Unazukionsen, Kurobe City (1); Fukumitsu-Horinji, Nanto City (1); Fukumitsu-Habu, Nanto City (1); Fukumitsu-Kawanishi, Nanto City (1); Fukumitsu-Yomekane, Nanto City (1); Kanjoji, Nanto City (2);

Kouzu, Nanto City (1); Minodani, Nanto City (1); Suganuma, Nanto City (1); Tatenogahara, Nanto City (1); Togamura-Toga, Nanto City (1); Anrakuji, Oyabe City (1); Hakkoden, Oyabe City (1); Ronden, Oyabe City (1); Fukuokamachi-Kono, Takaoka City (1); Fukuokamachi-Mainoya, Takaoka City (1); Fukuokamachi-Saimyoji, Takaoka City (1); Zukawa, Takaoka City (1); Yasukawa, Tonami City (1); Aoyanagishin, Toyama City (1); Arimine, Toyama City (1); Ashu, Toyama City (1); Ioridani, Toyama City (1); Joyama, Toyama City (1); Katakake, Toyama City (9); Tochiori, Toyama City (6); Numanomata, Toyama City (1); Manganji, Toyama City (2); Sannokuma, Toyama City (1); Kanayamadani, Uozu City (1); Tozo, Uozu City (1); Inamura, Kamiichi-machi, Nakaniikawa-gun (2); Iori, Kamiichi-machi, Nakaniikawa-gun (1); Kakizawa, Kamiichi-machi, Nakaniikawa-gun (1); Orito, Kamiichi-machi, Nakaniikawa-gun (2); Tachi, Kamiichi-machi, Nakaniikawa-gun (1); Ikeda, Tateyama-machi, Nakaniikawa-gun (1); Yotuyao, Tateyama-machi, Nakaniikawa-gun (1); Hosono, Asahi-machi, Shimoniikawa-gun (1); Ishitani, Asahi-machi, Shimoniikawa-gun (1); Yamazaki, Asahi-machi, Shimoniikawa-gun (1); Aoshima, Nyuzen-machi, Shimoniikawa-gun (1). **Fukui Pref.**: Ichihashi, Tsuruga City (1); Makidani, Minamiechizen-cho, Nanjo-gun (1); Ozohara, Echizen-cho, Nyu-gun (1). **Gifu Pref.**: Kiyomicho-Makigahora, Takayama City (1); Kiyomicho-Kamikojima, Takayama City (1); Hagiwaracho, Gero City (6); Maki, Shirakawa-mura, Ohno-gun (1); Shokawacho-Nonomata, Takayama City (1); Shimonokiri, Takayama City (1); Takasucho-Hirugano, Gujo City (1); Furukawacho-Onocho, Hida City (1); Miyagawacho-Nishishinobi, Hida City (1); Fukuchi, Yaotsu-cho, Kamogun (1). **Aichi Pref.**: Kiriyamacho, Okazaki City (4); Mizuwakare, Okazaki City (1); Kandonocho, Toyota City (1). **Shiga Pref.**: Takatsuki, Takatsuki-cho, Ika-gun (1); Ryuo-cho, Kamou-gun (1)

H. sibthorpioides

2n = 24 **Shizuoka Pref.**: Yachiyocho, Shizuoka City (1); Minamimatsuno, Fujikawa-cho, Ihara-gun (1). **Gifu Pref.**: Makino, Minokamo City (1). **Okinawa Pref.**: Kurashiki, Okinawa City (1)

H. yabei

2n = 24 **Kanagawa Pref.**: Honmoku, Yokohama City (1). **Shizuoka Pref.**: Tadaki, Mikkabi-cho, Inasa-gun (1). **Toyama Pref.**: Shirakawa, Himi City (1); Unazukionsen, Kurobe City (1); Fukumitsu-Yoshimi, Nanto City (1); Fuchumachi-Konagasawa, Toyama City (1); Gofuku, Toyama City (1); Hara, Toyama City (1); Kodani, Toyama City (1); Kurehayama, Toyama City (1). **Gifu Pref.**: Hagiwaracho, Gero City (1). **Shiga Pref.**: Hikonejoshi, Hikone City (1); Tachibana, Hikone City (1); Kinomoto-cho, Ika-gun (1). **Kyoto Pref.**: Tangocho-Oyamamachi, Kyotango City (1). **Kagoshima Pref.**: Oyamada, Kagoshima City (1); Nishino, Minamitane-cho, Kumage-gun (1); Shiroyamacho, Kagoshima City (2)